Physicochemical endolysin, holin and endolysin-holin properties

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Introduction

An accurate protein formulation is required in order to assure an effective protein delivery, specially when it comes to therapeutic products and pharmaceuticals, as proteins are not fully adaptable to changes in their environment. Under stress, proteins could no longer be functional if their stability requirements are not fulfilled. When working with fused proteins, such as the endo-holin complex, several factors need to be taken into consideration, factors that can lead towards an adequate formulation, which will make the complex resistant to any external factors that could affect the protein's structure or activity.

Isoelectric point and hydrophobicity

The endo-holin complex is made from two distinct proteins, with different stability properties, which are an endolysin and a holin. When fused, the protein does not act in a predictable way. Modeling programs were consulted in order to determine two of the six required characteristics: isoelectric point and hydrophobicity. Programs such as iTasser and Swissprot were used in order to determine said characteristics. These programs require the DNA coding sequence for the proteins, which we already have, and can theoretically predict several characteristics of the desired protein. All three proteins behave as hydrophilic and their isoelectric points are reported in **Table 1.1.**

Optimal temperature and pH range

Thermostability assays have already been performed on the endolysin, which determined the stability parameters of 20-40°C for temperature and 4-8 for pH, being 6 the optimal pH.[1] However, the holin is reported to be thermally unstable and cannot surpass thermal changes. As the endo-holin construct will contain said holin it is highly recommendable to include thermal protection inside the formulation proposal.[1,2] Also, pH sensitivity cannot be determined theoretically, so an approximation of 5 to 7 is made for the endo-holin construct. However, further experimental analysis should be performed in order to determine the specific optimal pH of the endo-holin protein.

Viscosity and pressure range

Several instructors, professors, and modelling programs were consulted for these parameters. However, a theoretical approach is not helpful at this stage of the project. Viscosity will rely on the solution's concentration and is not a fixed quantity when working with biological systems, as it increases proportionally to the denaturation of protein. if the protein is denatured or degraded, then the solution will increase its viscosity.[3]

Regarding pressure range there is no es viable method to accomplish the protein pressurization due to the aggressive temperature change method [4], mostly because the Holin does not accept these aggressive changes in its parameters.

Table 1.1 Physicochemical properties of endolysin, holin and endo-holin.

Property	**Holin**	**Endolysin**	**Endo-holin**
Isoelectric point	7.7	9.48	9.1
Hydrophobicity	Hydrophilic	Hydrophilic	Hydrophilic
Temperature range	37ºC	20~40 °C	37°C recommended
pH range	4 -10	4.0 - 8.0 (6.0 as optimal)	5-7 recommended. Further analysis are required.
Viscosity	Experimental analysis required	Experimental analysis required	Experimental analysis required
Pressure range	Experimental analysis required	Experimental analysis required	Experimental analysis required

References

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